A study of analysis PB1-F2 protein of Influenza Viruses A/H1N1pdm09, A/H3N2, and A/H5N1

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Abstrak

Latar belakang: *Protein PB1-F2 (polymerase basic 1-frame 2) adalah protein terbaru yang ditemukan pada virus Influenza dan telah terbukti berperan dalam induksi kematian sel dan patogenitas. Tujuan dari tulisan ini adalah untuk menganalisis faktor yang berhubungan dengan virulensi dan patogenesis yaitu variasi panjang asam amino dan mutasi N66S dari protein PB1-F2 pada virus Influenza A/H1N1pdm09, A/H3N2, dan A/H5N1.*

Metode: *Kami melakukan pencarian data yang relevan yaitu sekuens gen virus Influenza A/H1N1pdm09, A/H3N2, dan A/H5N1 dari Gen Bank National Center for Biotechnology Information (NCBI). Data yang digunakan adalah data sekuens nukleotida gen PB1 (polymerase basic1) virus influenza A/H1N1pdm09, A/H3N2, dan A/H5N1. Kemudian dilakukan analisis alignment untuk mengetahui variasi protein dan mutasi yang berhubungan dengan patogenitas dan virulensi.*

Hasil: *Hasil pencarian sekuens PB1-F2 yaitu terdapat 3262 Influenza A/H1N1pdm09, 7475 A/H3N2, dan 2551 influenza A/H5N1. Hasil analisis menunjukkan bahwa semua sekuens A/H3N2 dan A/H5N1 memiliki panjang yang penuh sebanyak 90 asam amino, kecuali influenza pandemi A/H1N1 2009 hanya memiliki panjang 87 asam amino. Kemudian, ditemukan mutasi yang berhubungan dengan virulensi yang ditunjukan dengan perubahan asam amino Asparagin (N) menjadi Serin (S) pada asam amino posisi 66. Mutasi tersebut terjadi pada Influenza A/H1N1pdm09 sebanyak 0.5%, 2.6% pada InfluenzaA/H3N1, dan Influenza A/H5N1 sebanyak 8.3%.*

Kesimpulan: *Ditemukan beberapa variasi panjang asam amino dan mutasi penting pada sekuens PB1-F2 dari inang dengan subtipe yang berbeda yaitu influenza A/H1N1pdm09, A/H3N2, dan A/H5N1 yang mengindikasikan seleksi spesifik karena introduksi dan adaptasi terhadap inang yang berbeda. Diperlukan penelitian lanjutan untuk lebih memahami variasi dan kontribusi protein PB1-F2 tersebut terhadap virulensi dan patogenitas virus Influenza yang saat ini sedang beredar.* ***(Health Science Journal of Indonesia 2016;7:7-12)***

Kata kunci: *Patogenesis, Virus Influenza, Protein PB1-F2*

Abstract

Background: Influenza virus PB1-F2 (*polymerase basic 1-frame 2*) protein is a novel protein previously shown to be involved in cell death induction, virulence, and pathogenesis. Here we analyze the factor contribute to virulence and pathogenesis which are length varibility and N66S mutation the PB1-F2 protein of Influenza virus A/H1N1pdm09, A/H3N2, and A/H5N1.

Methods. We conducted database search of Gen Bank National Center for Biotechnology Information (NCBI) for Influenza virus sequences. Data pertinent to this study is PB1 gene of A/H1N1pdm09, A/H3N2, and A/H5N1 Influenza viruses. We conducted the multiple alignments to determine the various length and important N66S mutations.

Results. The PB1-F2 sequences from the 3262 Influenza A/H1N1pdm09, 7475 A/H3N2, and 2551 Influenza A/H5N1 were studied. The analysis showed that all Influenza A/H3N2 and A/H5N1 carried the full length of 90 amino acids of PB2-F1 sequences, but the Influenza pandemic A/H1N1 2009 carried only 87 amino acids. In addition, the mutation indicates the presence of a significant correlation with the virulence shown by Serine at amino acids number 66 which replaces Asparagines (N66S). The mutation occurs in 0.5% of Influenza A/H1N1pdm09, 2.6% of Influenza A/H3N2, and 8.3% of Influenza A/H5N1.

Conclusion. Several varying lengths and important N66S mutations of PB2-F1 sequences from different host of Influenza A/H1N1pdm09, A/H3N2, and A/H5N1 were studied, indicating the positively selected mutations in specific subtype due to introduction and adaptation to different hosts. Further studies are required to understand the contribution of PB1-F2 proteins in virulence and pathogenesis of recent circulating influenza viruses. ***(Health Science Journal of Indonesia 2016;7:7-12)***

Keywords: Pathogenesis, Influenza virus, PB-F2 Protein

Influenza viruses cause substantial morbidity and mortality in humans worldwide and cause outbreaks of variable intensity annually.[1] Influenza A Viruses are mostly responsible for seasonal epidemics and global pandemics, and sustain the burden of disease attributable to influenza. It leads to pandemics, as in case of 1918 H1N1, 1957 H2N2, and 1986 H3N2 causing millions of death. In recent years, the influenza A/H1N1 pandemic in 2009 has caused more than 18,000 patients deaths in more than 200 countries.[2] Furthermore, highly pathogenic influenza A/H5N1 viruses have caused the potential epizootic and epidemic both in poultry and human in many countries, which is associated with high mortality (59%) and severe disease.[3]

The influenza A virus genome is made up of 8 separate segments of negative-sense single-stranded RNA (Ribonucleic acid) that encode 11 known proteins. The glycoproteins surface is well known as a important factor in evolution of Influenza viruses; however among the eight segments, PB1 is the only segment that was exchanged in the pandemic viruses of 1957 and 1968.[4] In addition, the new PB1 was discovered in influenza A/H1N1 pandemic 2009.[2] The 11th protein, the PB1-F2, has been identified and characterized by Chen et al.[5] This non-structural protein is a small mitochondria protein which is highly conserved in influenza isolates, expressed especially by influenza A virus.[6] It is located at an alternate open reading frame near the 5' end encoded by the +1 reading frame of the PB1 segment and consists of up to 87 or 90 residue protein (fig. 1).[5] Several studies have found that this PB1-F2 protein plays important roles in pathogenesis and virulence of influenza virus infection during epidemic and pandemic.[6-8]

The influence of PB1-F2 on viral pathogenes in mouse models infection was first demonstrated in recombinant viruses that possessed the PB1 segment from influenza A/PR/8/34 (H1N1) virus (PR8) and the 7 remaining genomic segments from influenza A/WSN/33 (H1N1) virus (WSN). In this experiment, they generated influenza viruses knocked out for the expression of PB1-F2 protein, resulting no effect on viral replication in tissue culture but decreased virus pathogenicity and mortality in mice.[6] McAuley *et al*[9] have characterized the effects of PB1-F2 in mice enhances the development of secondary bacterial pneumonia from *Streptococcus pneumoniae* and thus decreases survival. Reverse genetic study by Pena *et al* have showed that the PB1-F2 modulates A/H3N1 virus replication, virulence, and innate immune response in pigs.[10]

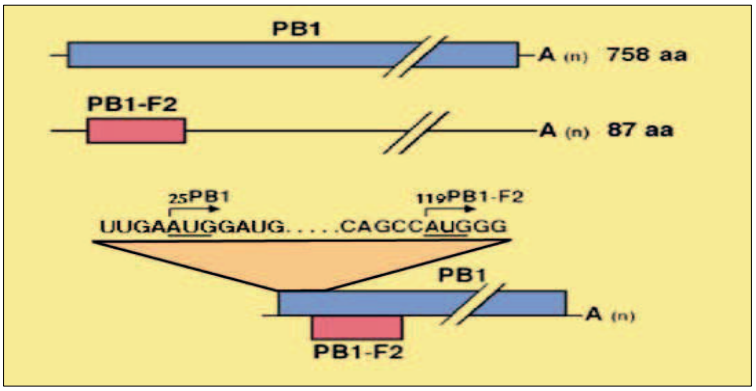

Figure 1. The bicistronic PB1 gene segment of Influenza A Virus.21 The two ORFs in influenza A RNA segment 2 that encode the polymerase PB1 and the PB1-F2 protein.

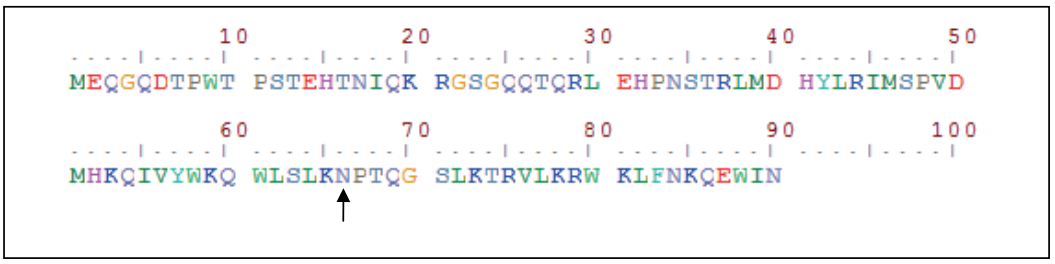

Figure 2. PB1-F2 protein alignment. The size of PB1-F2 polypeptides ranges from 79 to 101 amino acids (aa). The arrow shows the single amino acid mutation Asparagines to Serine at position 66 (N66S).

A study using the recombinant 1997 Hong Kong H5N1 and 1918 pandemic H1N1 viruses shows that the virulence of the viruses is associated with the presence of serine (S) at position 66 instead of asparagines (N) of the polymerase protein PB1-F2 (fig.2), which is induce increased levels of cytokines in the lungs of infected mice.[7] Position 66 is located at the a-helical structure of PB1-F2 in the mitochondria, which could affect PB1-F2 interactions with proteins constituting the permeability transition pore complex, such as the ANT3 (adenine nucleotide translocator 3) and VDAC1 (voltage-dependant anion channel 1).[11,12] It is involved in targeting leukocytes such as TNF-αTo undergo apoptosis PB1-F2 protein acts through the interaction with mitochondrial permeability transition pore complex, and play a role in the down regulation of the host immune response to influenza virus infections.[12] This interaction promotes the permeabilization of the mitochondria and can potentially increase the induction of apoptosis by PB1-F2.[7,11]

Here we conducted a study to analyze the PB1-F2 protein of Influenza A/H1N1pdm09, A/H3N2, and A/H5N1 in countries especially focusing on the variety of length and important N66S mutation.

METHODS

Data Sources

We conducted NCBI[13] sequences database search starting from 1997 until 2015. The selection was based on an extensive search for Influenza A/H1N1pdm09, A/H3N2, and A/H5N1 viruses whose PB1 nucleotide sequences were shown to alternatively translate into a PB1-F2 gene.

Data Selection

To determine the existence of PB1-F2 in influenza A/H1N1pdm09 isolates; 2304 of human, 23 avian, 934 swine, and 1 environment sequences were selected between 2009 and 2015. The PB1-F2 sequences of influenza A/H3N2 were also selected from 1997-2015 which are 6197 of human, 199 of avian, 1074 of swine, 5 of environment. In addition, we included all sequences of 236 human, 2240 avian, 25 swine, and 50 environment sequences of influenza A/H5N1 between 1997 and 2015 from public database. All sequences contained a start codon (ATG) at position 95-97 in the PB1 gene, which translated to Methionine (M) and marked as a beginning of PB1-F2 ORF, whereas the stop codon (TGA) is marked at position 365-367 of the PB1 gene.

Data Analysis

The sequences of Influenza viruses A/H1N1pdm09, A/H3N2, and A/H5N1 and were aligned and compared using BioEdit version 7.0.8.0 (Ibis Biosciences, USA) [14] to identify the variety of length size and important mutations.

RESULTS

Analysis of Influenza A/H1N1pdm09 PB1-F2 protein

PB1-F2 sequences of a total 3262 Influenza A/H1N1pdm09 isolated between 2009 and 2015 were analyzed. The sequences were isolated 2304 from human, 23 from avian, 934 from swine, and 1 from environment. Hundred percent of human and environment sequences, 17 percent (4 of 23) avian sequences, and 87% (819 of 934) had an N-terminal truncated by the presence of a stop codon at position 11, 58, and 83 PB1-F2 protein of 87 amino acid length. It is likely to contribute to survival or transmission in the natural avian host.[15] In addition, among the influenza A/H1N1pdm09, only avian (30%) and swine (1%) of PB1-F2 sequences had mutation asparagines (N) to serine (S) at position 66. (Table 1)

Analysis of Influenza A/H3N2 PB1-F2 protein

The PB1-F2 sequences of Influenza A/H3N2 derived from human, avian, swine, and environment which are contain full-length 90 amino acids residues. Our analysis of 7475 sequences of PB1-F2 Influenza A/H3N2 subtype strain; 41 (0.6%) of 6197 human isolates, 144 (72%) from 199 Avian isolates, 10 (0.9%) of 1074 swine isolates, and 2 (40%) out of 5 of environment isolates were carriying the N66S mutation. In total we found about 197 (2.7%) of mutation N66S among 7475 sequences of PB1-F2 influenza A/H3N2

Analysis of Influenza A/H5N1 PB1-F2 protein

The human, avian, swine, and environment H5N1 viruses covered the entire full-length ORF (90 amino acid residues). In this study, we found 13 (5.5%), 181 (8.35%), and 18 (36%) sequences of human, avian, and environment of A/H5N1 viruses respectively have serine (S) mutations instead of asparagines (N) (table 1, fig 3). However, none of mutation of N66S was found at swine isolates. The analysis revealed that the mutation N66S was only present in 212 (8.3%) of 2551 Influenza A/H5N1 strain.

Table 1. PB1-F2 variant and an N66S mutation in various host

Subtype	Host	Total No. of Strain	No of Strain Carrying N66S mutation (%)	Length of AA (90)	Length of AA (87)
A/H1N1pdm09	Human	2304	0 (0)	0	2304
	Avian	23	7 (30)	19	4
	Swine	934	10 (1)	115	819
	Environment	1	0 (0)	0	1
A/H3N2	Human	6197	41 (0.6)	6197	0
	Avian	199	144 (72)	199	0
	Swine	1074	10 (0.9)	1074	0
	Environment	5	2 (40)	5	0
A/H5N1	Human	236	13 (5.5)	236	0
	Avian	2240	181 (8.3)	2161	0
	Swine	25	0 (0)	25	0
	Environment	50	18 (36)	50	0

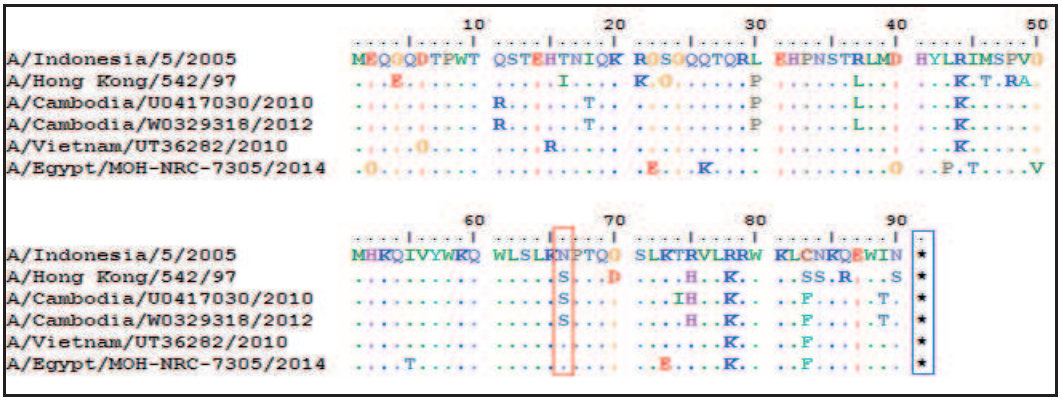

Figure 3. Comparative alignment of PB1-F2 protein between human A/H5N1 representatives sequences. All strains contain a full length PB1-F2 of 90 residues. The amino acid residue related to pathogenesis at the position 66 is red squared and the stop codon is blue squared. Alignment is generated by BioEdit version 7.0.9 software.

DISCUSSION

Influenza viruses cause annual epidemics and occasional pandemics in the world. Many of the molecular markers are predicted to be associated with virulence, pathogenicity, and adaptation to a human host or to the generation of a pandemic virus, as seen in 1918 H1N1, highly pathogenic H5N1, A/H1N1pdm09 viruses. The PB1 gene proven has been one of the segments that reasserted and created the pandemic strains of 1957 and 1968.[15] In addititon, PB1-F2 protein derived from PB1 gene is the most recently discovered protein of influenza viruses that might be crucial in the generation of a new devastating pandemic strain. We find the variation mutation on several Influenza A isolates. Mutation associated to virulence is indicated by Serine instead of Asparagines (N66S) that occurs in variety of host such as human, avian, swine, and environment isolates. Majority of that mutation (N66S) is occurs at avian host in A/H1N1pdm09 and A/H3N2, and also in environment in A/H5N1 strain. That mutation is known to be associated with virulence and pathogenesis of influenza A infection. It is one of the molecular markers associated with pathogenicity, as indicated by the mutation a single amino acid at position 66 (N66S). This finding has been proven through some experiments conducted by Conenello et all[11]. They knocked out the expression of PB1-F2 in mice, which showed a significant loss in pathogenicity, indicating that PB1-F2 is found to play an important role in virulence. In another study, a single amino acid at position 66 in PB1-F2 from highly virulent viruses increases pathogenicity in mice and interferes with the immune response. It causes apoptosis of immune cells, which contributes to a decrease in antigen presentation and the adaptive immune response. The finding also provides evidence

that PB1-F2 contributes to the high virulence in highly pathogenic viruses which is also found in the 1918 H1N1 virus.[16] The substitution of serine for asparagine at position 66 of PB1-F2 enhances replication of the virus in vitro models and reduces the IFN response in infected avian monocytes. In an in vivo mouse model, this substitution enhances pathogenicity, replication, and neurotropism.[12] The increase in viral pathogenicity of N66S mutant is possibly due to inhibition of type I interferon and proinflammatory responses in monocytic cells.[12] The presence of mutation serine to asparagine at position amino acids 66 may be important when influenza viruses cross species barriers or when new pandemic strains are generated by reassortment.[17]

The different subtype of Influenza A has varying length of the PB1-F2 protein. An analysis of the length of PB1-F2 protein of Influenza A/H5N1 strain by Chakrabarti and Parischa[18] indicated that a complete protein was found in 96% of the strain, suggesting that PB1-F2 is positively selected in these strain and its definitely essential for the virus. Eventhought the influenza A/H5N1 viruses are high pathogenic which is indicated by high mortality rate, the mutation carrying Serine instead of asparagines at position 66 only occur in 13 (5.5%) out of 236 human isolates. This mutation is only present in Hongkong and Cambodia isolates, but not in any isolates from Indonesian, Egypt, or Vietnam which are the countries who are affected by A/H5N1 with a high mortality rate. It seems that, though containing a full length PB1-F2 ORF and mutation of serine which is known as the marker of virulence at the position number 66, the factor of virulence and pathogenesis is still questioning in H5N1 viruses.

Interestingly, the sequences of Influenza A/H1N1pdm09 contain a truncated PB1-F2, possibly this could happened because high viral titers cause the death of the host cell, thus preventing newer viruses from continuing further replication.[19] In addition, after introduction of viruses into mammalian hosts such as humans or swine, however, the protein often becomes truncated during adaptation and evolution.[20] During the evolution of H1N1 viruses over time in humans, a stop codon at position 58 in the PB1-F2 amino acid sequence appeared around 1950, and has been retained in the human H1N1 lineage since its re-emergence in 1977.[15] Similarly, appearance of truncations has been identified at different positions including position 58, such that 25% of swine PB1-F2 sequences in NCBI lack the C-terminal portion of the protein that contains mitochondrial targeting sequence which has several functions to mediate PB1 binding and promote inflammation.[15] Recently, A/H1N1pdm09 has emerged in 2009. It is indicated that it contains a reassortment virus composed of genes derived from avian, swine, and human viruses. However, it does not express a full length PB1-1 F2 which is important for rapid systemic viral dissemination of HPAIV in birds.[12] Consequently, the Influenza A/H1N1pdmo9 is not as highly pathogenic as the Hong Kong 1997 H5N1 strains or the 1918 H1N1 pandemic strain. [12,15,16]

In conclusion, in this study, we found several varying length of PB1-F2 protein from various host of Influenza A/H1N1pdm09, A/H3N2, and A/H5N1. A full length protein is expressed by the Influenza A/H3N2 and A/H5N1; however, in the recent A/H1N1 pandemic strain 2009, especially in the human host, the PB1-F2 protein is non-functional due to the a terminal truncation which are indicating positively selected in specific subtype due to introduction and adaptation into different host. Moreover, the analysis also determined the point mutation of amino acid 66 of PB1-F2 revealed the importance of this protein in the virulence of human A/H1N1pdm09 pandemic virus, A/H3N2, and A/H5N1. A further study to understand the contribution of PB1-F2 proteins in virulence and pathogenesis of recent circulating influenza viruses, especially the potentially pandemic virus is required.

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